Supporting Information

Squaramide-based supramolecular materials for 3D cell culture of human induced pluripotent stem cells and their derivatives

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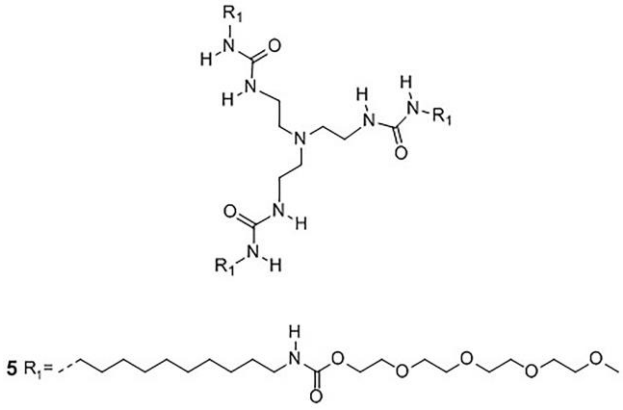
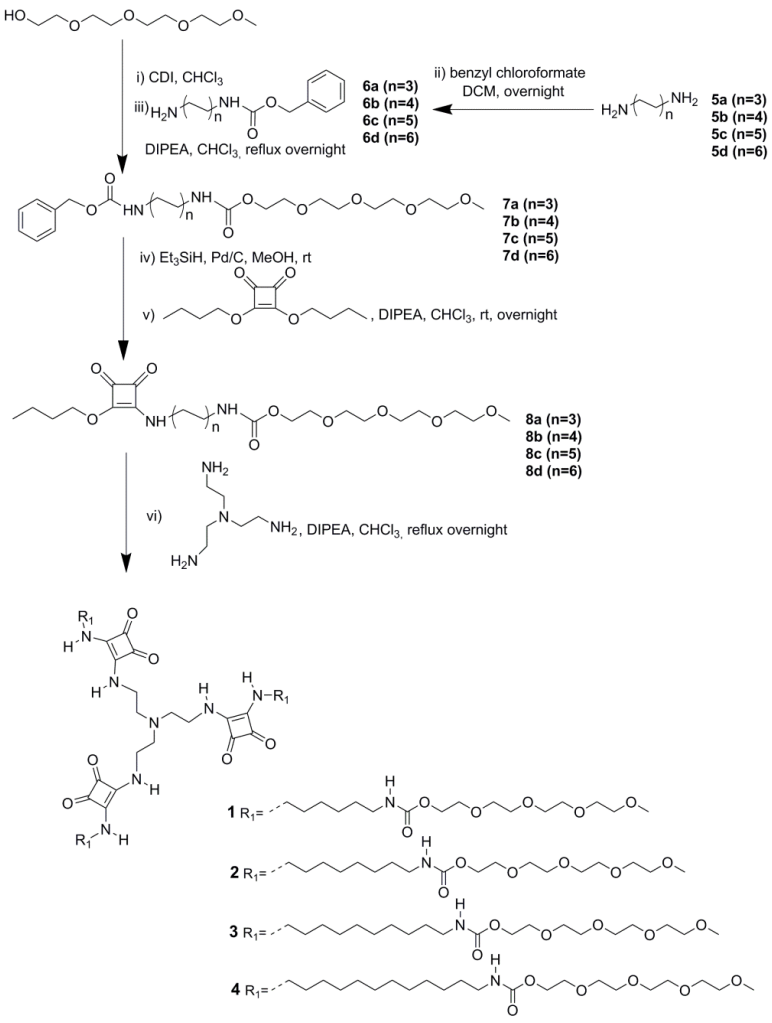
S1 Experimental Details

S1.1 Structure of monomer 5

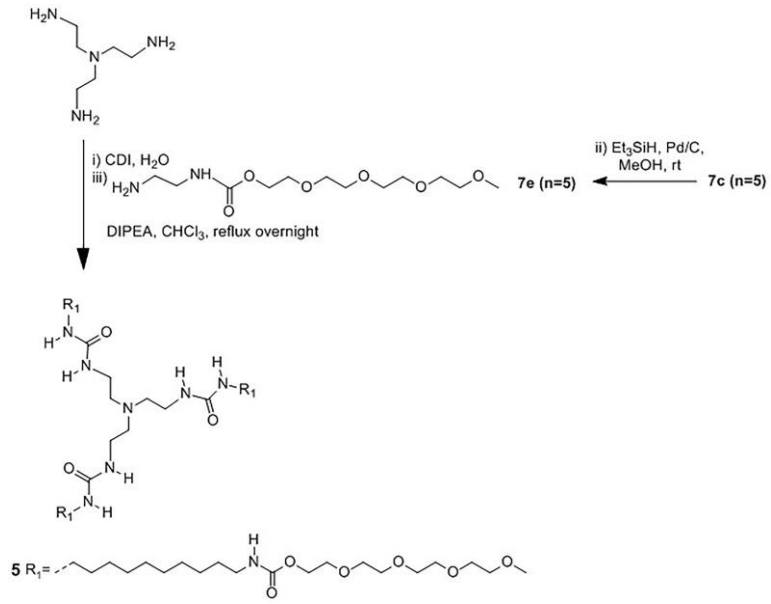


Scheme S1. Tripodal urea-based monomer **5**.

S1.2 Synthetic routes of monomers 1-5



Scheme S2. Synthetic route of monomers **1-4**.



Scheme S3. Synthetic route of monomer **5**.

S1.3 Synthetic procedures

Synthesis of compounds 6a-d

To a solution of benzyl chloroformate (**a**: 2.94 g, 17.23 mmol; **b**: 1.89 g, 11.08 mmol; **c**: 1.98 g, 11.61 mmol; **d**: 1.11 g, 6.51 mmol) in CH_2Cl_2 (100 mL) a stirred solution of the corresponding 1,n-alkyldiamine (**5a** (n = 6): 10.00 g, 86.14 mmol; **5b** (n = 8): 8.03 g, 55.66 mmol; **5c** (n = 10): 10.00 g, 58.05 mmol; **5d** (n = 12): 6.49 g, 32.39 mmol) in CH_2Cl_2 (100 mL) was added dropwise over 2 hours at 0°C. The reaction mixture was allowed to stir overnight at room temperature. The solution was then concentrated by rotary evaporation and EtOAc was added. The mixture washed 3x with water and the aqueous layers were discarded. The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuum to obtain a white solid (**6a**) without further purification. For **6b-d**, when 1 M HCl was added, a white precipitate formed in the organic layer. The solid was collected by filtration, washed with EtOAc and used without further purification.

Compound 6a

Yield: 2.67 g, 62%. 1H-NMR (CD_3OD, 400 MHz): 7.34-7.28 (m, 5H), 5.06 (s, 2H), 3.12-3.09 (m, 2H), 2.64-2.60 (m, 2H), 1.51-1.43 (m, 4H), 1.35-1.32 (m, 4H). ^{13}C-NMR (CD_3OD, 100

MHz): 158.85, 138.44, 129.21, 128.90, 128.71, 67.24, 42.28, 41.66, 33.33, 30.81, 27.56, 27.38. MALDI-TOF-MS: m/z calc: 250.17, found: 250.47 $[M+H]^+$.

Compound 6b

Yield: 2.31 g, 75%. ^{1}H-NMR (CD_3OD, 400 MHz): 7.34-7.27 (m, 5H), 5.06 (s, 2H), 3.12-3.07 (m, 2H), 2.94-2.89 (m, 2H), 1.69-1.63 (m, 2H), 1.53-1.46 (m, 2H), 1.41-1.31 (m, 8H). ^{13}C-NMR (CD_3OD, 100 MHz): 158.49, 138.07, 129.13, 128.60, 128.48, 66.82, 41.28, 40.32, 30.41, 29.64, 28.09, 27.20, 26.92, 26.90. MALDI-TOF-MS: m/z calc: 278.20, found: 278.59 $[M+H]^+$.

Compound 6c

Yield: 2.77 g, 78%. ^{1}H-NMR (CD_3OD, 400 MHz): 7.34-7.27 (m, 5H), 5.06 (s, 2H), 3.11-3.08 (m, 2H), 2.93-2.89 (m, 2H), 1.67-1.62 (m, 2H), 1.50-1.46 (m, 2H), 1.40-1.32 (m, 12H). ^{13}C-NMR (CD_3OD, 100 MHz): 158.47, 138.07, 129.11, 128.56, 128.39, 66.81, 41.35, 40.34, 30.46, 30.08, 29.96, 29.90, 29.74, 28.13, 27.35, 27.00. MALDI-TOF-MS: m/z calc: 306.23, found: 306.65 $[M+H]^+$.

Compound 6d

Yield: 1.39 g, 64%. ^{1}H-NMR (CD_3OD, 400 MHz): 7.34-7.27 (m, 5H), 5.06 (s, 2H), 3.12-3.07 (m, 2H), 2.92-2.89 (m, 2H), 1.67-1.61 (m, 2H), 1.49-1.46 (m, 2H), 1.41-1.30 (m, 16H). ^{13}C-NMR (CD_3OD, 100 MHz): 158.90, 138.50, 129.43, 128.75, 128.69, 67.24, 41.79, 40.77, 30.91, 30.66, 30.64, 30.60, 30.48, 30.39, 30.22, 28.58, 27.82, 27.45. MALDI-TOF-MS: m/z calc: 334.26, found: 334.67 $[M+H]^+$.

Synthesis of compounds 7a-d

Tetraethyleneglycol monomethyl ether (**a**: 1.03 g, 4.95 mmol; **b**: 1.30 g, 6.24 mmol; **c**: 1.50 g, 7.20 mmol; **d**: 1.30 g, 6.24 mmol) was first activated with 1,1′-carbonyldiimidazole (**a**: 0.88 g, 5.44 mmol; **b**: 1.11 g, 6.85 mmol; **c**: 1.28 g, 7.92 mmol; **d**: 1.11 g, 6.87 mmol) for 1 hour at room temperature. Subsequently, **6a-d** (**6a**: 1.49 g, 5.94 mmol; **6b**: 2.08 g, 7.49 mmol; **6c**: 2.65 g, 8.64 mmol; **6d**: 2.50 g, 7.49 mmol), DIPEA (**a**: 1.7 mL, 9.90 mmol; **b**: 2.2 mL, 12.48

mmol; **c**: 2.5 mL, 14.41 mmol; **d**: 2.2 mL, 12.48 mmol) and $CHCl_3$ (15 mL) were added to the reaction mixture and refluxed overnight. Once the reaction was finished, CH_2Cl_2 (15 mL) was added and washed with H_2O (30 mL). The combined aqueous fractions were then back-extracted 3x with CH_2Cl_2 (3 x 30 mL). The combined organic fractions were dried with $MgSO_4$, prior to removal of the solvent *in vacuo*. The crude product was purified by silica column chromatography using a CH_2Cl_2/EtOAc gradient (20-50 vol% EtOAc). The product was evaporated to dryness by rotary evaporation to obtain a white solid **7a-d** and placed in a vacuum oven overnight.

Compound 7a

Yield: 1.46 g, 61%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.29-7.24 (m, 5H), 5.20 (br s, 1H), 5.11 (br s, 1H), 5.03 (s, 2H), 4.15-4.13 (m, 2H), 3.76-3.58 (m, 12H), 3.50-3.48 (m, 2H), 3.32 (s, 3H), 3.13-3.05 (m, 4H), 1.45-1.38 (m, 4H), 1.27-1.21 (m, 4H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 156.23, 136.42, 128.35, 128.01, 127.84, 71.69, 70.37, 70.31, 70.27, 69.43, 66.34, 63.60, 58.79, 40.65, 40.57, 29.62, 26.02. LC-MS: t = 6.66 min, m/z: 485.27 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 484.28, found: 506.81 $[M+Na]^+$.

Compound 7b

Yield: 2.26 g, 71%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.33-7.25 (m, 5H), 5.06 (s, 2H), 4.98 (br s, 1H), 4.89 (br s, 1H), 4.18-4.16 (m, 2H), 3.65-3.60 (m, 12H), 3.53-3.51 (m, 2H), 3.34 (s, 3H), 3.17-3.08 (m, 4H), 1.47-1.42 (m, 4H), 1.25 (s, 8H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 156.17, 136.38, 128.52, 128.17, 127.98, 71.59, 70.27, 70.21, 70.17, 69.35, 66.24, 63.46, 59.03, 40.76, 40.67, 29.59, 28.83, 26.32. LC-MS: t = 7.37 min, 513.33 m/z $[M+H]^+$. MALDI-TOF-MS: m/z calc: 512.31, found: 534.88 $[M+Na]^+$.

Compound 7c

Yield: 2.45 g, 63%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.35-7.29 (m, 5H), 5.08 (s, 2H), 4.83 (br s, 1H), 4.77 (br s, 1H), 4.21-4.19 (m, 2H), 3.68-3.62 (m, 12H), 3.55-3.53 (m, 2H), 3.37 (s, 3H), 3.20-3.11 (m, 4H), 1.49-1.43 (m, 4H), 1.32-1.24 (m, 12H). ^{13}C-NMR ($CDCl_3$, 100 MHz):

156.50, 136.75, 128.35, 128.20, 128.16, 71.99, 70.66, 70.61, 70.57, 69.75, 66.62, 63.86, 59.06, 41.18, 41.10, 30.00, 29.48, 29.28, 26.78. LC-MS: t = 8.09 min, m/z: 541.33 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 540.34, found: 562.82 $[M+Na]^+$.

Compound 7d

Yield: 1.70 g, 48%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.35-7.27 (m, 5H), 5.08 (s, 2H), 4.87 (br s, 1H), 4.77 (br s, 1H), 4.21-4.18 (m, 2H), 3.70-3.61 (m, 12H), 3.54-3.52 (m, 2H), 3.37 (s, 3H), 3.19-3.10 (m, 4H), 1.48-1.43 (m, 4H), 1.26-1.23 (m, 16H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 156.53, 136.78, 129.01, 128.61, 128.18, 72.02, 70.70, 70.64, 70.60, 69.79, 66.66, 63.89, 59.13, 41.22, 41.14, 30.04, 29.60, 29.35, 26.83. LC-MS: t = 8.80 min, 569.40 m/z: $[M+H]^+$. MALDI-TOF-MS: m/z calc: 568.37, found: 590.87 $[M+Na]^+$.

Synthesis of compounds 8a-d

Compound **7a-d** (**7a**: 1.33 g, 2.75 mmol; **7b**: 1.74 g, 3.40 mmol; **7c**: 1.14 g, 2.11 mmol; **7d**: 1.17 g, 2.05 mmol) was dissolved in anhydrous MeOH (10 mL), and Pd/C (**a**: 29.80 mg, 0.28 mmol; **b**: 36.18 mg, 0.34 mmol; **c**: 22.35 mg, 0.21 mmol; **d**: 22.35 mg, 0.21 mmol) was added. The solution was degassed with nitrogen, prior to the dropwise addition of triethylsilane (**a**: 4.4 mL, 27.50 mmol; **b**: 5.4 mL, 34.00 mmol; **c**: 3.4 mL, 21.10 mmol; **d**: 3.3 mL, 20.50 mmol). The addition of triethylsilane resulted in the formation of an effervescent solution and once the reaction was complete (as demonstrated by TLC), the solution was filtered through Celite to remove the remaining Pd/C. The filtrate was concentrated by rotary evaporation and afterwards, a gentle stream of nitrogen gas. The dried product was redissolved in CH_2Cl_2 (15 mL). 3,4-Dibutoxy-3-cyclobutene-1,2-dione (**a**: 0.65 mL, 3.03 mmol; **b**: 0.81 mL, 3.74 mmol; **c**: 0.50 mL, 2.32 mmol; **d**: 0.49 mL, 2.26 mmol) and DIPEA (**a**: 0.95 mL, 5.50 mmol; **b**: 1.1 mL, 6.80 mmol; **c**: 0.74 mL, 4.22 mmol; **d**: 0.71 mL, 4.10 mmol) were added to the reaction mixture and stirred at room temperature overnight. Subsequently, CH_2Cl_2 (15 mL) was added and washed with H_2O (30 mL). The aqueous fractions were back-extracted 3x with CH_2Cl_2 (3 x 30 mL). The organic fractions were combined and dried with $MgSO_4$, prior to removing the

solvent *in vacuo*. The crude product was further purified by silica gel column chromatography using a CH_2Cl_2/EtOAc gradient (10-50 vol% EtOAc). The product was concentrated by rotary evaporation to provide an oil (**8a-d**) that was further dried in a vacuum oven overnight.

Compound 8a

Yield: 1.09 g, 73%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.14 (br s, 1H), 4.71-4.68 (m, 2H), 4.17-4.15 (m, 2H), 3.64-3.59 (m, 12H), 3.52-3.50 (m, 2H), 3.38-3.35 (m, 2H), 3.33 (s, 3H), 3.14-3.09 (q, 2H), 1.76-1.71 (m, 2H), 1.59-1.56 (m, 2H), 1.47-1.30 (m, 8H), 0.95-0.89 (m, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 189.71, 182.75, 177.52, 172.47, 156.64, 73.45, 71.87, 70.54, 70.49, 70.46, 70.43, 69.62, 63.82, 58.99, 44.65, 40.70, 32.03, 30.43, 29.77, 26.10, 25.90, 18.67, 13.70. LC-MS: t = 6.20 min, 503.20 m/z $[M+H]^+$. MALDI-TOF-MS: m/z calc: 502.29, found: 524.81 $[M+Na]^+$.

Compound 8b

Yield: 1.23 g, 68%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 7.47 (br s, 1H), 5.10 (br s, 1H), 4.60-4.57 (m, 2H), 4.08-4.03 (m, 2H), 3.53-3.48 (m, 12H), 3.41-3.39 (m, 2H), 3.30-3.24 (m, 2H), 3.22 (s, 3H), 3.01-2.96 (q, 2H), 1.66-1.60 (m, 2H), 1.48-1.45 (m, 2H), 1.35-1.16 (m, 12H), 0.84-0.78 (m, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 189.33, 182.88, 176.97, 172.30, 156.27, 72.99, 72.84, 71.57, 70.23, 70.19, 70.16, 70.12, 69.30, 63.47, 58.66, 44.52, 40.64, 31.72, 30.29, 29.57, 28.81, 28.73, 26.32, 26.02, 18.37, 13.41. LC-MS: t = 6.83 min, m/z: 531.33 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 530.32, found: 552.87 $[M+Na]^+$.

Compound 8c

Yield: 0.81 g, 69%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.59 (br s, 1H), 4.91 (br s, 1H), 4.74-4.71 (m, 2H), 4.20-4.18 (m, 2H), 3.67-3.62 (m, 12H), 3.55-3.52 (m, 2H), 3.41-3.36 (m, 2H), 3.36 (s, 3H), 3.16-3.11 (q, 2H), 1.79-1.75 (m, 2H), 1.61-1.57 (m, 2H), 1.48-1.26 (m, 16H), 0.97-0.94 (m, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 189.77, 182.84, 177.42, 172.66, 156.63, 73.42, 72.02, 70.68, 70.63, 70.61, 70.58, 69.75, 63.91, 59.11, 44.99, 41.13, 32.15, 30.77, 30.04,

29.53, 29.34, 29.23, 26.83, 26.50, 18.80, 13.83. LC-MS: t = 7.60 min, m/z: 559.27 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 558.35, found: 580.91 $[M+Na]^+$.

Compound 8d

Yield: 0.62 g, 52%. ^{1}H-NMR ($CDCl_3$, 400 MHz): 6.23 (br s, 1H), 4.87 (br s, 1H), 4.75-4.67 (m, 2H), 4.21-4.19 (m, 2H), 3.68-3.63 (m, 12H), 3.56-3.53 (m, 2H), 3.44-3.41 (m, 2H), 3.37 (s, 3H), 3.17-3.12 (q, 2H), 1.62-1.56 (m, 2H), 1.49-1.25 (m, 22H), 0.98-0.95 (m, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz): 189.66, 182.70, 177.40, 172.43, 156.40, 73.36, 71.89, 70.56, 70.51, 70.47, 69.66, 63.79, 59.00, 44.89, 41.02, 32.01, 30.66, 29.92, 29.49, 29.45, 29.23, 29.12, 26.72, 26.37, 18.66, 13.68. LC-MS: t = 8.32 min, m/z: 587.27 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 586.38, found: 608.55 $[M+Na]^+$.

Synthesis of compounds 1-5

Compound **8a-d** (**8a**: 0.52 g, 1.04 mmol; **8b**: 0.49 g, 0.92 mmol; **8c**: 0.64 g, 1.15 mmol; **8d**: 0.32 g, 0.54 mmol) was dissolved in $CHCl_3$ (15 mL). Tris(2-aminoethyl)amine (**a**: 46 µL, 0.31 mmol; **b**: 40 µL, 0.27 mmol; **c**: 52 µL, 0.35 mmol; **d**: 24 µL, 0.16 mmol) and DIPEA (**a**: 181 µL, 1.04 mmol; **b**: 160 µL, 0.92 mmol; **c**: 200 µL, 1.15 mmol; **d**: 94 µL, 0.54 mmol) were added to the reaction mixture. The solution was refluxed overnight and purified by flash column chromatography on a C18 silica gel column using a gradient of 10-90% CH_3CN/H_2O over 35 minutes. The product was concentrated by rotary evaporation and lyophilized. The compounds were further purified by HPLC with UV detection and lyophilized to obtain the white compounds **1-4**.

For the synthesis of compound **5**, tris(2-aminoethyl)amine (12 µL, 0.08 mmol) was dissolved in water (5 mL) and stirred on an ice bath. Subsequently, 1,1'-carbonyldiimidazole (40 mg, 0.25 mmol) was added to the reaction mixture and stirred for 30 minutes. Another identical amount of 1,1'-carbonyldiimidazole was added and the reaction was stirred for another 30 minutes on an ice bath. Once the reaction was complete (as demonstrated by TLC-MS), the aqueous solution was lyophilized overnight and redissolved in $CHCl_3$. The

hydrogenated compound **7e** (107 mg, 0.26 mmol) derived from **7c** and DIPEA (45 µL, 0.26 mmol) were added to the reaction mixture and refluxed overnight. The product was purified by HPLC using mass detection and lyophilized overnight to obtain a white solid **5**.

Compound 1

Yield: 0.22 g, 51%. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.46 (br s, 3H), 7.27 (br s, 3H), 7.18 (br s, 3H), 4.03-4.01 (m, 6H), 3.55-3.40 (m, 54H), 3.23 (s, 9H), 2.96-2.93 (m, 6H), 2.70-2.68 (m, 6H), 1.49-1.26 (m, 24H). ^{13}C-NMR (DMSO-d_6, 100 MHz): 182.65, 168.27, 167.77, 156.42, 71.54, 70.07, 70.03, 69.98, 69.84, 69.16, 63.27, 58.31, 54.96, 43.53, 41.83, 40.40, 30.96, 29.57, 26.13, 25.85. LC-MS: t = 5.14 min, m/z: 1431.87 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 1430.80, found: 1452.69 $[M+Na]^+$.

Compound 2

Yield: 0.22 g, 54%. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.53 (br s, 3H), 7.34 (br s, 3H), 7.14 (br s, 3H), 4.04-4.01 (m, 6H), 3.58-3.40 (m, 54H), 3.23 (s, 9H), 2.96-2.91 (m, 6H), 2.73 (s, 6H), 1.53-1.21 (m, 36H). ^{13}C-NMR (DMSO-d_6, 100 MHz): 182.63, 168.28, 167.71, 156.45, 71.61, 70.14, 70.11, 70.07, 69.91, 69.25, 63.32, 58.30, 55.05, 43.66, 41.60, 40.52, 31.05, 29.98, 29.72, 29.08, 29.02, 26.60, 26.21. LC-MS: t = 5.93 min, m/z: 1515.67 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 1514.81, found: 1536.80 $[M+Na]^+$.

Compound 3

Yield: 0.30 g, 52%. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.49 (br s, 3H), 7.30 (br s, 3H), 7.18 (br s, 3H), 4.04-4.02 (m, 6H), 3.56-3.38 (m, 54H), 3.24 (s, 9H), 2.96-2.91 (q, 6H), 2.69 (s, 6H), 1.49-1.23 (m, 48H). ^{13}C-NMR (DMSO-d_6, 100 MHz): 182.59, 168.23, 167.68, 156.36, 71.52, 70.05, 70.02, 69.96, 69.82, 69.15, 63.22, 58.29, 54.97, 43.55, 41.63, 40.44, 30.97, 29.66, 29.28, 29.25, 29.02, 28.94, 26.53, 26.15. LC-MS: t = 6.89 min, m/z: 1599.87 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 1598.99, found: 1622.42 $[M+Na]^+$.

Compound 4

Yield: 0.11 g, 42%. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.61 (br s, 3H), 7.45 (br s, 3H), 7.16 (br s, 3H), 4.03-4.01 (m, 6H), 3.57-3.40 (m, 54), 3.23 (s, 9H), 2.95-2.90 (q, 6H), 2.64 (s, 6H), 1.51-1.21 (m, 60H). ^{13}C-NMR (DMSO-d_6, 100 MHz): 182.40, 182.27, 168.03, 167.45, 156.17, 71.33, 69.86, 69.83, 69.78, 69.64, 68.96, 63.03, 58.09, 54.80, 43.38, 41.43, 40.25, 30.76, 29.47, 29.14, 29.12, 28.86, 28.77, 26.35, 25.96. LC-MS: t = 7.81 min, m/z: 1684.73 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 1683.08, found: 1706.45 $[M+Na]^+$.

Compound 5

Yield: 0.065 g, 56%. ^{1}H-NMR (DMSO-d_6, 400 MHz): 7.19-7.16 (m, 3H), 6.21 (br s, 6H), 4.04-4.01 (m, 6H), 3.55-3.41 (m, 54H), 3.23 (s, 9H), 2.98-2.90 (m, 12H), 1.38-1.22 (m, 48H). ^{13}C-NMR (DMSO-d_6, 100 MHz): 158.87, 156.40, 71.54, 70.07, 70.04, 69.98, 69.84, 69.17, 63.25, 58.31, 54.46, 40.47, 30.17, 29.68, 29.33, 29.30, 29.14, 29.05, 26.74, 26.55. LC-MS: t = 6.86 min, m/z: 1443.5 $[M+H]^+$. MALDI-TOF-MS: m/z calc: 1443.0, found: 1444.43 $[M+H]^+$.

S2 Methods and Supporting Data

S2.1 Gel inversion tests and critical gelation concentration (CGC) determination

The CGC is defined as the minimum concentration of a given molecule to form a gel. The CGC of molecule **2** was determined by dissolving 2.5 mg (V_1), 3.0 mg (V_2), 3.5 mg (V_3), 4.0 mg (V_4), 4.5 mg (V_5) and 5.0 mg (V_6) of **2** in 500 µL of deionized water in glass vials (2 mL) to various concentrations. All samples were sonicated on a Branson 2510 Ultrasonic Cleaner bath at room temperature for 20 minutes at 298 K and left to stand overnight.

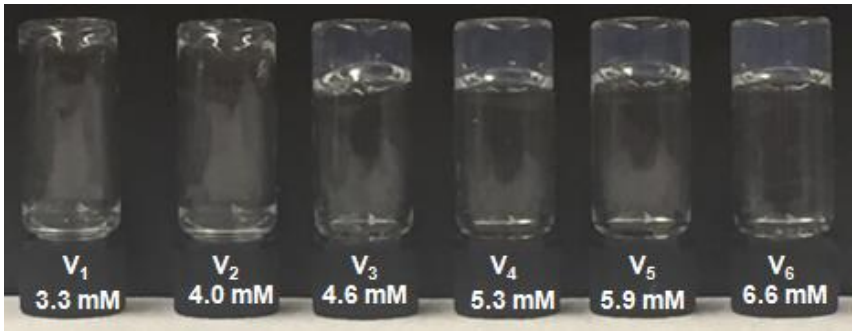


Figure S1. Gel inversion test of compound **2**.

The CGC of molecule **3** was also determined by dissolving 0.5 mg (V_1), 1.0 mg (V_2), 1.5 mg (V_3), 2.0 mg (V_4) and 2.5 mg (V_5) of **3** in 500 µL deionized water in a separate glass vials (2 mL) to various concentrations. All samples were sonicated on a Branson 2510 Ultrasonic Cleaner bath at room temperature for 20 minutes at 298 K.

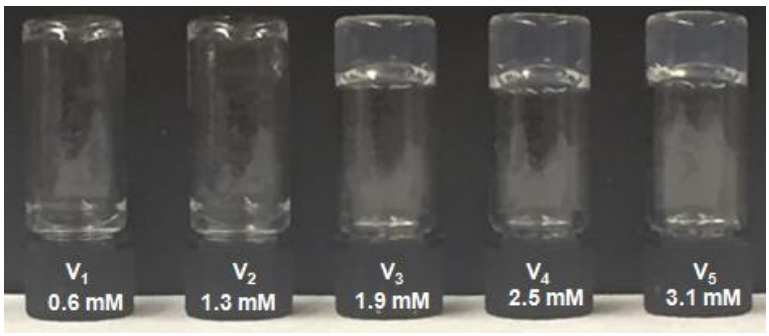


Figure S2. Gel inversion test of compound **3**.

S2.2 Oscillatory rheology

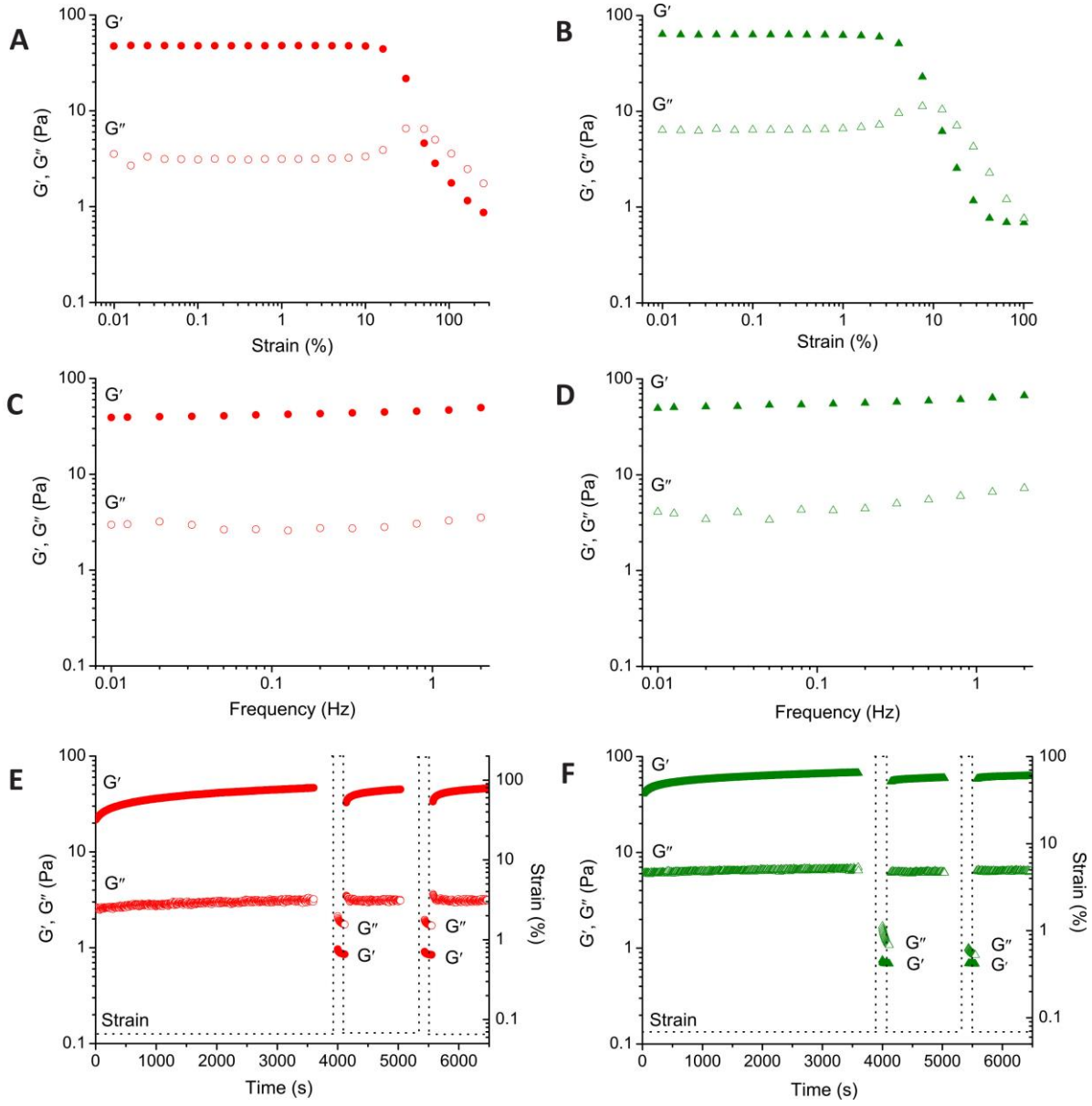


Figure S3. Oscillatory rheology measurements of hydrogels **2** (5.6 mM) and **3** (3.1 mM) in deionized water at 25 °C: Amplitude sweep (1 Hz) for hydrogels **2** (A) and **3** (B). Frequency sweep (0.05% strain) of hydrogels **2** (C) and **3** (D). Step strain measurements (1 Hz) for hydrogels **2** (E) and **3** (F), the absence of data between the application of high strain is due to the acquisition of a frequency sweep (from 0.01 to 2 Hz, $\gamma = 0.05\%$).

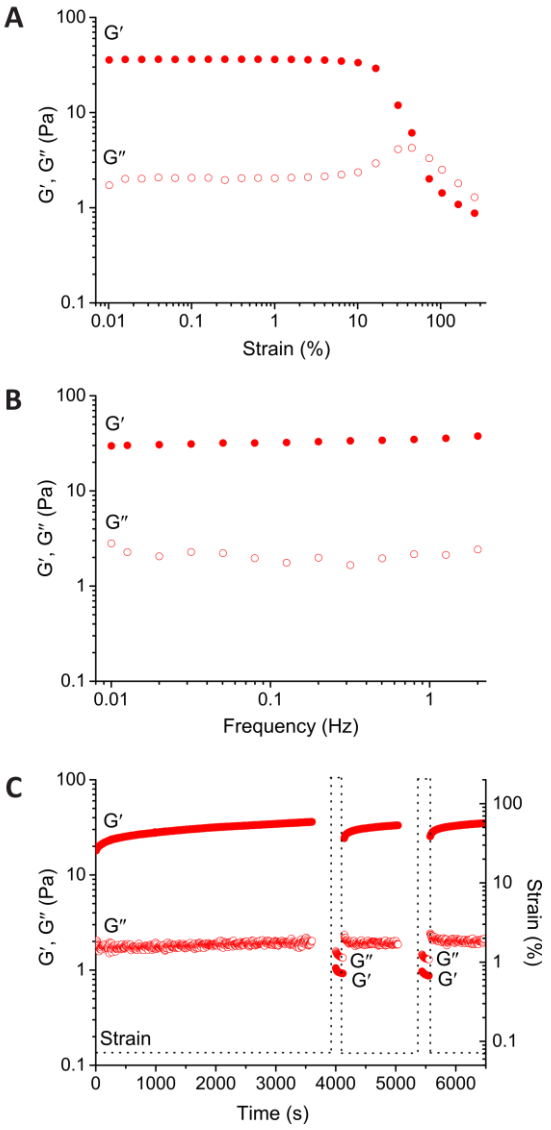


Figure S4. Oscillatory rheology measurements of the hydrogel **2** (5.6 mM) in PBS at 37 °C: (A) Amplitude sweep (f = 1 Hz). (B) Frequency sweep (γ = 0.05%). (C) Step strain measurements (f = 1 Hz). The absence of data before the application of high strain is due to the acquisition of a frequency sweep (from 0.01 to 2 Hz, γ = 0.05%).

S2.3 Cryo-electron microscopy (cryo-TEM).

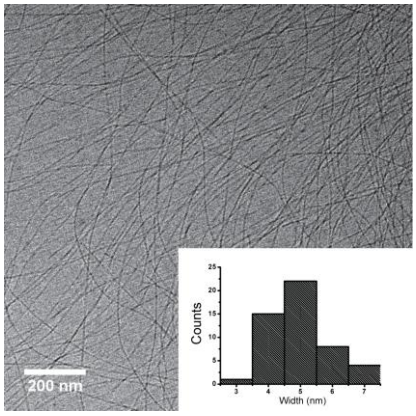


Figure S5. Cryo-TEM image of hydrogel **2** (5.6 mM) prepared by 20 minutes sonication in an ultrasonic bath and left to stand overnight. Insert: Histograms of width distribution of the fibers for a sample size of N = 50.

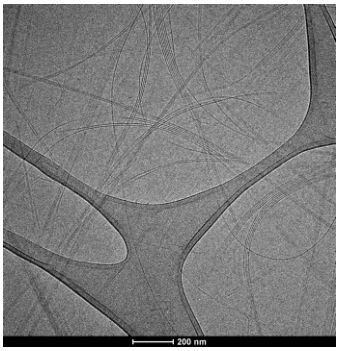


Figure S6. Cryo-TEM image of a solution **5** (3.1 mM) in deionized water prepared by 20 minutes sonication in an ultrasonic bath and left to stand overnight.

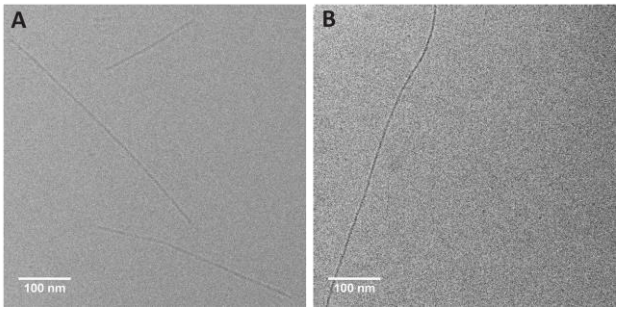


Figure S7. (A) Cryo-TEM image of a diluted solution of **2** (1.5×10^{-5} M) in deionized water and (B) diluted solution of **3** (1.5×10^{-5} M) prepared by 20 minutes sonication in an ultrasonic bath and left to stand overnight.

S2.4 Small angle X-ray Scattering (SAXS)

The experimental SAXS profile and the form factor model to describe it is given in Figure 2F and Figure S8. In the low-*q* regime the scattering profiles decay with a powerlaw slope of approximately unity, which is typical for one-dimensional objects. The experimental data was modelled using a form factor developed for flexible cylinders, with a fixed $\rho_{solvent} = 9.37 \cdot 10^6$ A^{-2}. From the model, we obtain values for the cross-sectional radius of the fibers, r_{cs}, their electron length density, ρ_{cyl}, and kuhn length, l_K (see Table S1). Several form factors were tested, including those of stiff homogeneous and flexible homogeneous cylinders (the latest best describe the data and was therefore selected). The slope in the low *q*-regime showed a value of 1.71 (Figure S8) and it was used for the determination of the $I_{cs}(q)$ (Figure S9). We extract the cross-sectional mass per unit length, M_L, from the height of the $I_{cs}(q)$ plateau according to

$$\frac{d\Sigma(q)}{d\Omega} = I(q) = \frac{\pi}{q} I_{cs}(q) \quad (1)$$

$$M_L = \frac{I_{cs}(0)}{c\Delta\rho_M^2} \quad (2)$$

with the electron length density difference per mass, $\Delta\rho_M$ (extracted from the fitting curves in SasView, where $\Delta\rho_M = \Delta(\rho_{cyl}-\rho_{solv})$.

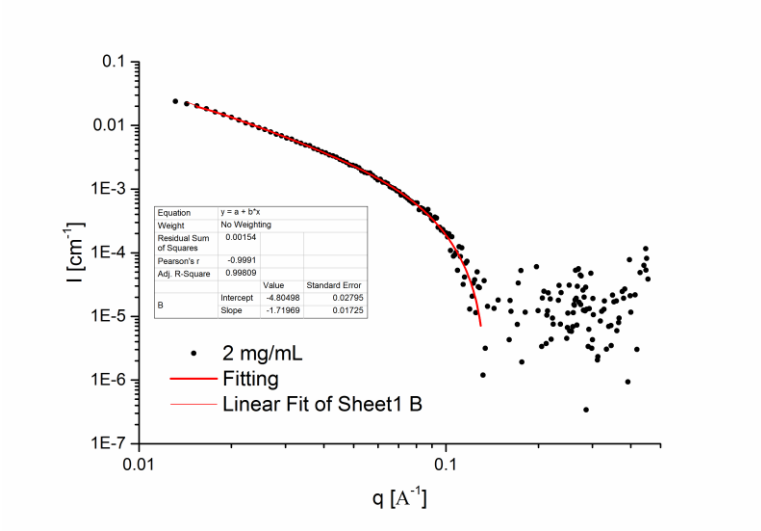


Figure S8. Scattering profile of **3** (2 mg mL^{-1}). Black dots represent experimental data; red line represents form factor model for flexible cylinders. Slope was determined to be ~ 1.71 in the low *q*-regime (blue line).

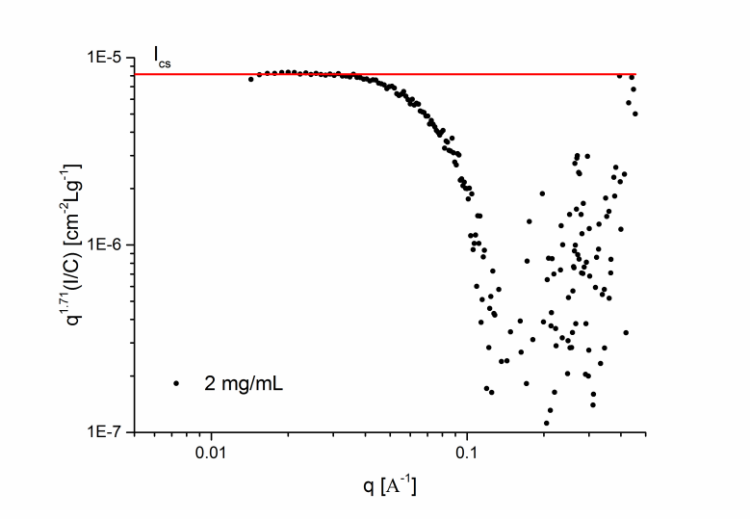


Figure S9. $I_{cs}(q)$ determination plot of the scattering profile in Figure 2F. The $I_{cs}(q)$ plateau ($0.0143 \leq q \leq 0.0383$ $Å^{-1}$) is indicated by a red line.

Table S1. Structural parameters extracted from Holtzer representation of SAXS profiles.

Sample	$\Delta\rho_{cyl}$ ($Å^{-2}$)	$\Delta\rho_{sol}$ ($Å^{-2}$)	$\Delta\rho_M$ (cm g^{-1})	I (cm^{-2} L g^{-1})	I_{cs} (0) (cm^{-2})	M_L (g/nm)	Mole c/nm	R_{cs} (nm)	Kuhn L (nm)
2 mg/mL	9.04 $\times 10^6$	9.37 $\times 10^6$	3.27 $\times 10^9$	8.15 $\times 10^2$	1.04 $\times 10^3$	2.42 $\times 10^{-21}$	0.92	2.608	6.591

S2.5 UV-Vis Spectroscopy

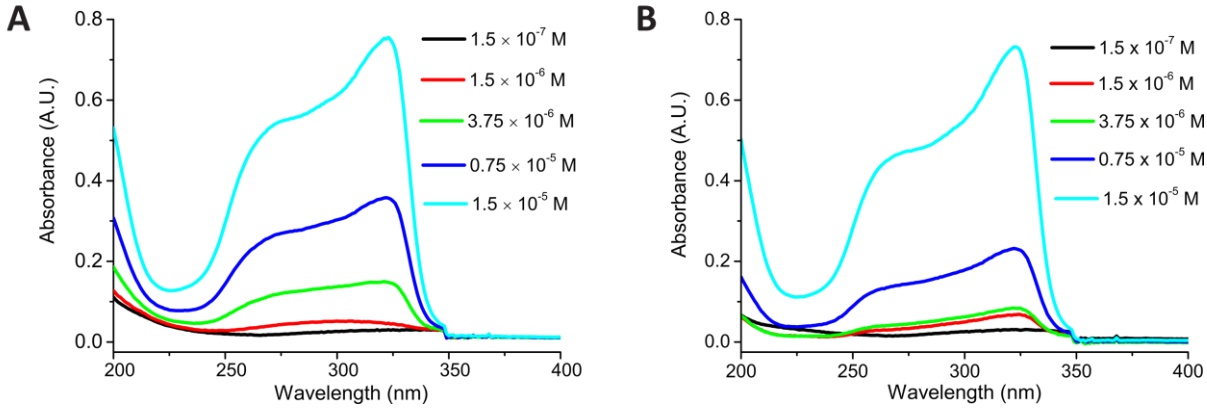


Figure S10. Concentration-dependent UV-Vis spectra of **2** (A) and **3** (B) in deionized water.

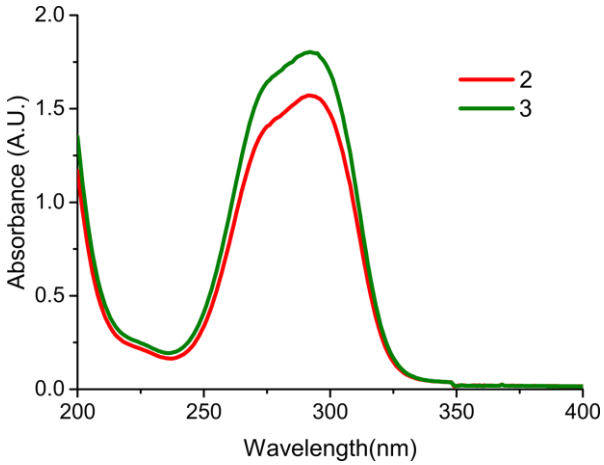


Figure S11. UV-Vis spectra of **2** and **3** (1.5×10^{-5} M) in HFIP/H_2O (v/v, 1:1).

S2.6 Fourier Transform Infrared (FTIR) spectroscopy

Table S2 Infrared spectra assignment of vibrational modes from 3700-1500 cm^{-1} for the various peaks indicated in Figure 3C of freeze-dried samples **1-3**.

	Sample **1**	Sample **2**	Sample **3**
ν(N-H)	3317 cm^{-1} (carbamate) 3164 cm^{-1} (squaramide)	3317 cm^{-1} (carbamate) 3165 cm^{-1} (squaramide)	3315 cm^{-1} (carbamate) 3167 cm^{-1} (squaramide)
ν(C-H)	2931 cm^{-1} (antisym) 2861 cm^{-1} (sym)	2925 cm^{-1} (antisym) 2854 cm^{-1} (sym)	2918 cm^{-1} (antisym) 2850 cm^{-1} (sym)
ν(C=O)	1719 and 1693 cm^{-1} (carbamate) 1654 and 1639 cm^{-1} (squaramide)	1719 and 1692 cm^{-1} (carbamate) 1644 cm^{-1} (squaramide)	1720 and 1689 cm^{-1} (carbamate) 1652cm^{-1} (squaramide)
Ring breathing	1799 cm^{-1}	1799 cm^{-1}	1799 cm^{-1}

S2.7 MTT assays

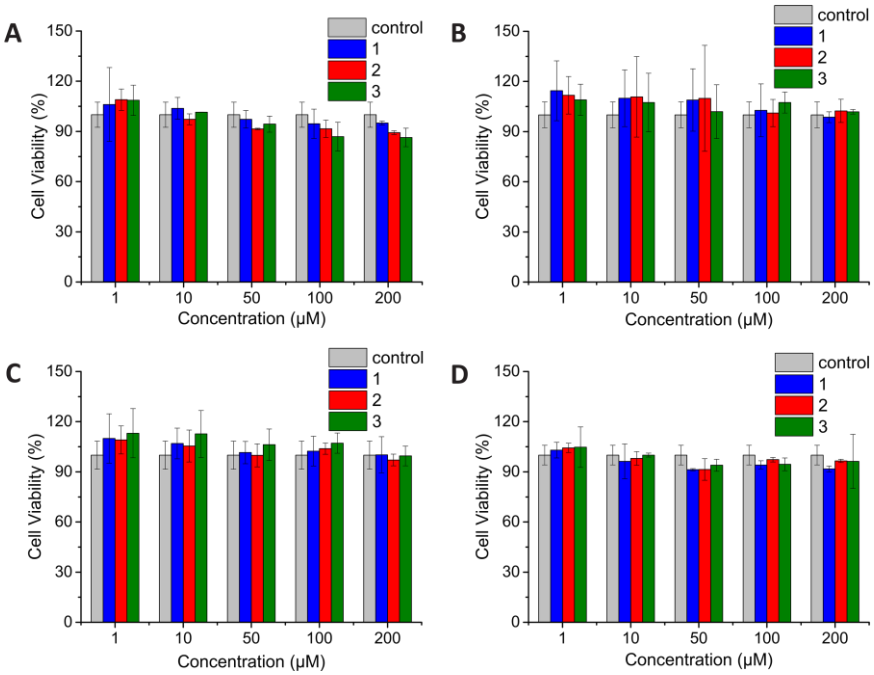


Figure S12. Results of the MTT cytotoxicity test for monomers **1-3** ranging in concentration (1-200 μM) with NIH 3T3 cells under various conditions (N = 3): (A) in deionized water after 24 h. (B) in deionized water after 72 h. (C) in PBS after 24 h. (D) in PBS after 72 h.

S2.8 Cell encapsulation

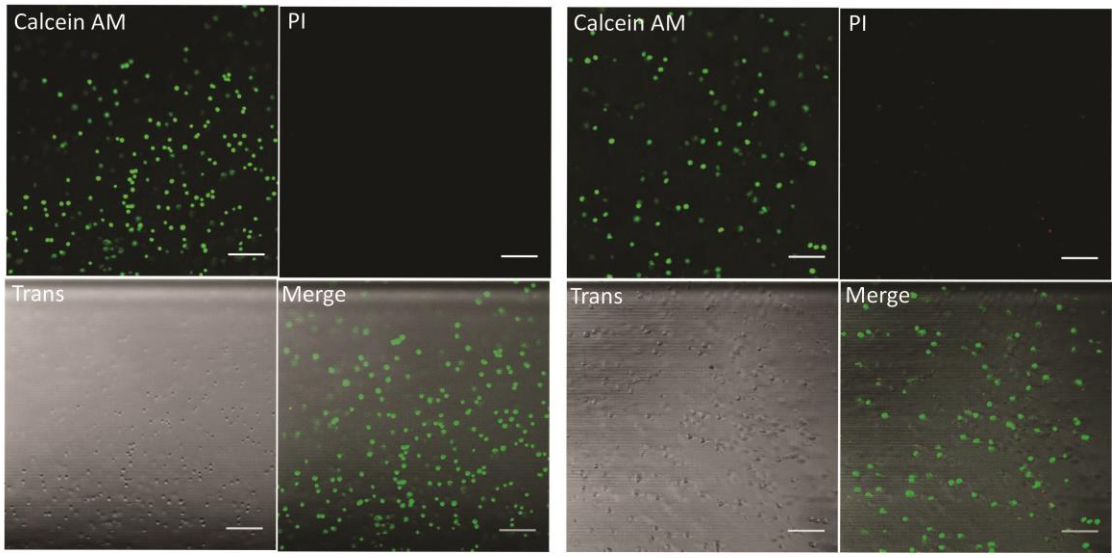


Figure S13. Confocal microscopy images of NIH 3T3 cells encapsulated in a hydrogel of **2** (5.6 mM) in 3D: after 2 h incubation (*left*); after 48 h incubation (*right*) (green: viable cells, red: dead cells). Scale bar 200 µm.

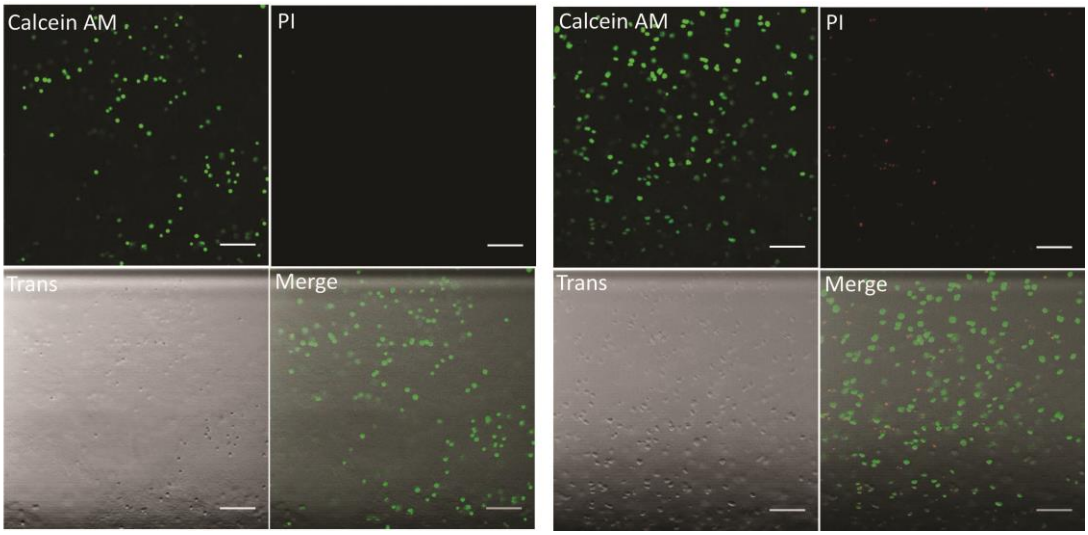


Figure S14. Confocal microscopy images of NIH 3T3 cells encapsulated in a hydrogel of **3** (3.1 mM): after 2 h incubation (*left*); after 48 h incubation (*right*) (green: viable cells, red: dead cells). Scale bar 200 µm.

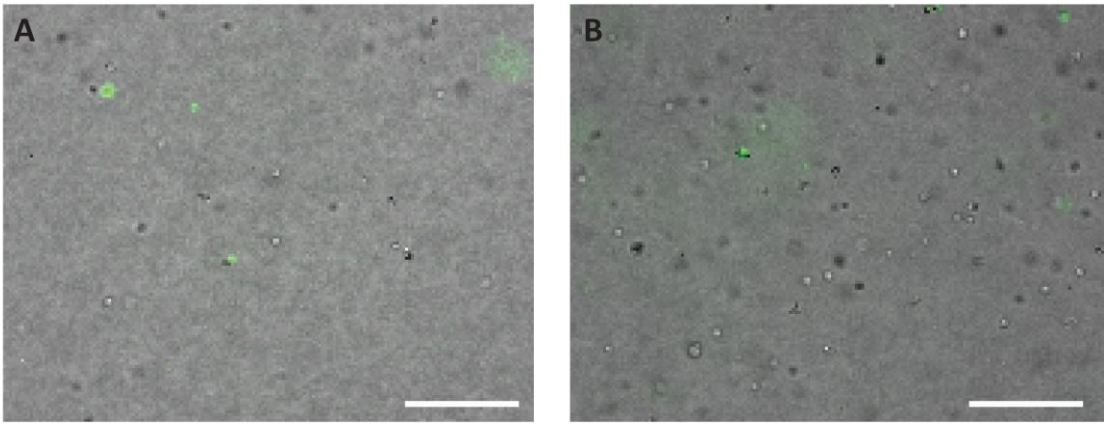


Figure S15. 3D cell culture of hiPSC-derived ECs encapsulated in the hydrogel **3** (3.1 mM): (A) Representative images of hiPSC-ECs seeded in hydrogel at low density (5×10^6 cells/mL) or (B) high density (2×10^7 cells/mL) and cultured for 24 h. Dead cells were detected using NucGreen® Dead reagent (ThermoFisher). Scale bar 200 µm.

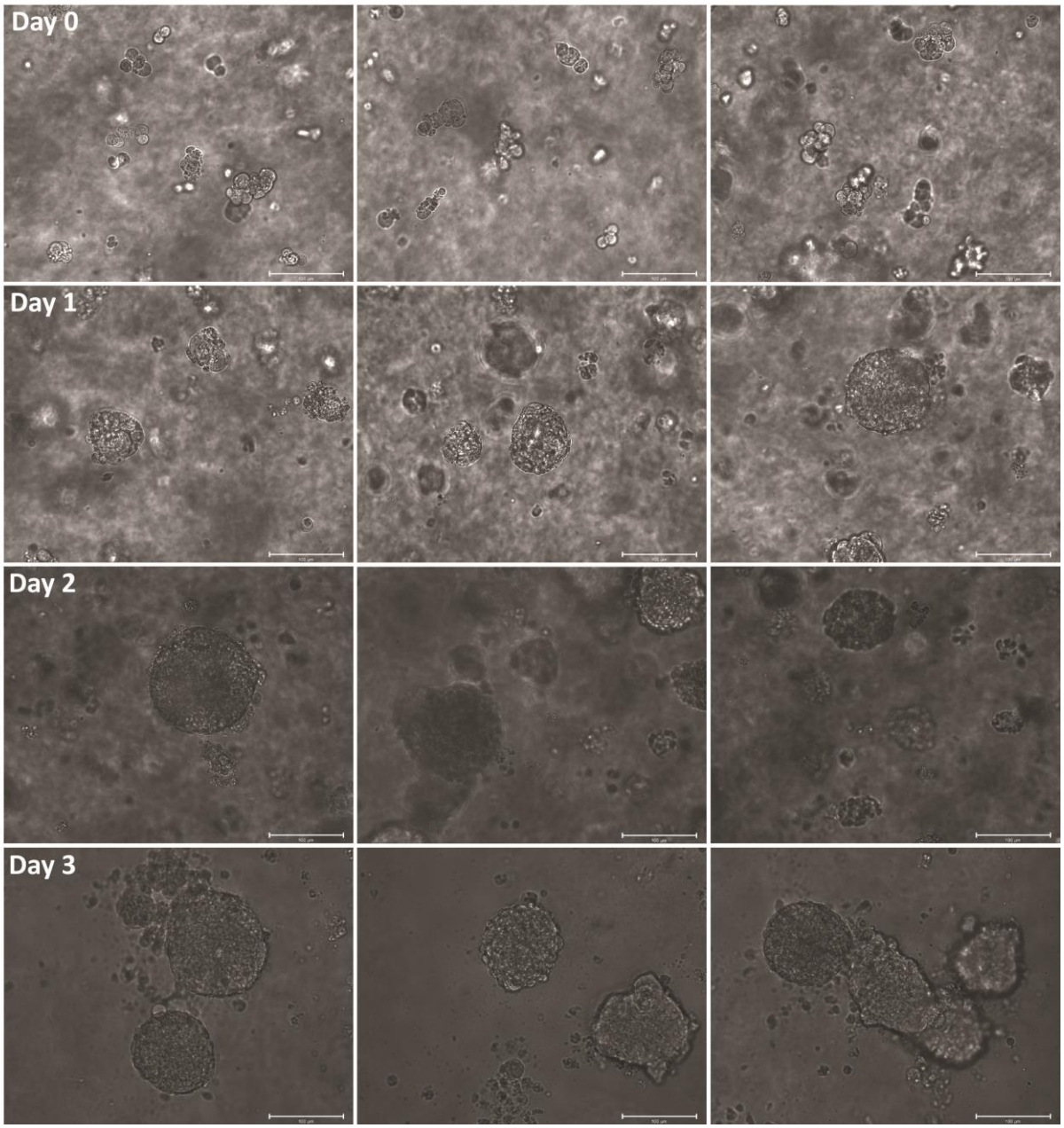


Figure S16. Representative images of hiPSCs in hydrogel **3** (3.1 mM) just after seeding, after 24 h, 48 h and 72 h of culture. Scale bar 100 µm.

S2.9 FACS analysis

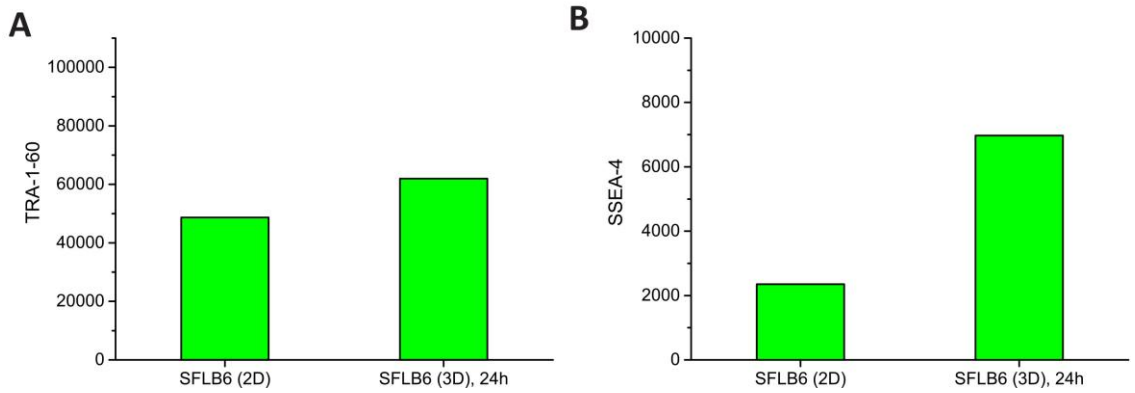


Figure S17. 3D cell culture of hiPSCs encapsulated in hydrogel **3** (3.1 mM): The values of mean fluorescence intensity (MFI) of the positive cell populations in the expression level of TRA-1-60 (A) and SSEA-4 (B).